§ 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner stated that with regard to claim 44, the recitation of the term "regenerating", it is unclear as to whether the regeneration refers to re-manufacturing of tissue *de novo* or the repair of tissue and the metes and bounds of the term cannot be adequately established. For examination purposes, the Examiner interpreted "regenerating" as repair.

Applicants respectfully traverse the Examiner's rejection and maintain that the plain meaning of "regenerating" means to form again, to generate or produce anew, as in regenerating by a new growth of tissue. The plain meaning of the term "repair" includes both restoring to a sound or healthy state and replacement of destroyed cells or tissues by new formation. Accordingly, the term "regenerating" has defined metes and bounds. Therefore, applicants maintain that Claims 1-3, 5-16, 18-25, 27-34, 36-49 and 53-55 are definite and distinctly claim the subject matter of the claimed methods.

Accordingly, applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 112, second paragraph.

Claims 1, 12, 23, 32, 39, 44 and claims dependent thereon

Claims 1, 12, 23, 32, 39, 44 and claims dependent thereon have been rejected under 35 U.S.C. § 112, second paragraph as being indefinite and unclear for the recitation of the term "effective".

The Examiner asserts that one of skill would not understand the metes and bounds of this term because the actual amount that is effective may vary according to the subject.

Applicants respectfully traverse the Examiner's rejection and maintain that the term effective is definite and clear, even though actual amounts may vary per subject (as is the case for most therapeutics). The subject specification provides clear guidance for one of skill in the art to understand what constitutes an effective amount of autologous BM-MNCs; specifically, the specification, *inter alia* at page 10, lines 1-3, states that an effective amount of approximately $1x10^5$ to $1x10^{10}$ cells, preferably $1x10^7$ to $1x10^8$ cells are delivered per

injection site.

Accordingly, one of skill in the art would know that the term "effective" amount has clear and definite metes and bounds. Therefore, applicants maintain that Claims 1, 12, 23, 32, 39, 44 and claims dependent thereon are definite and distinctly claim the subject matter of the claimed methods.

Accordingly, applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 112, second paragraph.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-3, 5-16, 18-25, 27-34, 36-49 and 53-55 have been rejected under 35 U.S.C. § 112, first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner asserts that the specification has not adequately described the steps which are necessary for the formation of new blood vessels because the claims are directed to the administration of a general pool of BM-MNC, will [sic] the cell type required for the invention, namely the EPCs, is not taught.

The Examiner asserts that the prior art teaches differentiation of bone marrow cells into endothelial cells through the stimulation of endothelial progenitor cells (EPC), found in the bone marrow, by cytokines and growth factors such as VEGF and once the EPCs are differentiated into endothelial cells, blood vessels will form. The Examiner further asserts that the addition of cytokines and growth factors is not claimed. The Examiner questions whether the presence of additional cell types may be deleterious. The Examiner asserts that one of skill would not know whether the administration of BM-MNC would adequately function in the manner described, because of alleged cytokine/growth factor requirements, the presence of adequate amounts of EPCs within the BM-MNCs and unknown effects of other cell types on BM-MNC administration to a specific site. The Examiner concludes that it would require undue experimentation by one of skill to practice the invention commensurate in scope with the claims.

Applicants respectfully traverse the above rejection and maintain that the specification as filed provides an adequate written description to enable one of skill in the art to practice the claimed methods.

Applicants' claimed method, as recited in claim 1, is: "[a] method of forming new blood vessels in tissue in a subject which comprises: a) isolating autologous bone marrow-mononuclear cells from the subject; and b) transplanting locally into the tissue an effective amount of the autologous bone-marrow mononuclear cells, resulting in formation of new blood vessels in the tissue."

Applicant's method does not require purification of EPCs from the BM-MNCs. The Examiner's attention is respectfully directed to the subject specification at page 11, lines11-13, the specification specifically states that "... it is not essential that EPCs be purified for use in the present invention. Isolation of BM-MNCs and transplantation thereof provides the desired beneficial effect." (Emphases added)

Moreover, the specification at page 11, lines 20-22, states that BM-MNCs without purification of EPCs might be a sufficient and even more effective cellular source for therapeutic neovascularization. (Emphases added) The specification at page 21, first paragraph, describes that BM-MNCs were shown to contain various cell types, including monocytoid cells and lymphoid cells, which according to Prockop (1997) are the cell types in which EPCs are believed to be present. The subset of adult BM-MNCs used in the claimed methods do in fact differentiate into EPCs and are incorporated into the capillary EC network. Therefore, administration of EPCs purified from BM-MNCs is not necessary in the practice of the claimed methods. In addition, the mixture of cells in the BM-MNCs work cooperatively with each other as feeder cells and a greater number of EPCs develop, as described on page 12, lines 9-11. Therefore, the presence of other cell types is advantageous, rather than deleterious. The Examples demonstrate the formation of EPCs developing from BM-MNCs and neovascular formation from BM-MNCs, confirming that the administration of BM-MNCs adequately function in the manner described and claimed.

Other advantages of the claimed methods of local transplantation of BM-MNCs include increasing the density of EPCs which develop from the BM-MNCs and the

elimination of graft-versus-host diseases which occur after bone marrow transplantation (*See,* Specification, page 13, lines 13-29) and avoidance of potential systemic side effects as compared to systemic infusion (*See,* page 37, lines 23-25).

With respect to the culture medium used for the isolated BM-MNCs, the specification, *inter alia* at page 9, lines 11-14 and page 8, lines 21-24, describes the use of any complete medium, e.g., Iscove's modified Dulbecco medium, for culturing for up to four weeks before transplantation. (Emphasis added) The specification states that the cells may be cultured with growth factors, e.g., vascular endothelial growth factor (*See,* page 9, lines 13-14) or bovine pituitary extract as an EC growth supplement (*See,* page 20, lines 17-20). Moreover, in a preferred embodiment, fresh bone-marrow mononuclear cells are used for transplantation, as stated in the specification at page 9, lines 8-9, without culturing.

Therefore, one of skill is adequately guided by the subject specification to practice the claimed invention commensurate in scope with the claims without undue experimentation. Accordingly, Claims 1-3, 5-16, 18-25, 27-34, 36-49 and 53-55 are fully enabled by the specification as filed.

Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw the rejection of these Claims under 35 U.S.C. §112, first paragraph.

Rejections under 35 U.S.C. § 102(b)

Kobayashi et al.

Claims 1-8, 10, 12-13, 15-16, 18-21, 23-28, 30, 32-34, 36-37, 39-40, 42, 44-45, 47-49 and 53-54 have been rejected under 35 U.S.C. § 102(a), as being anticipated by Kobayashi et al. (J. Surgical Res. 2000 Apr; 89(2):189-95).

Anticipation can only be established by a single prior art reference which discloses each and every element of the claimed invention. *Structural Rubber Prods. Co. v. Park Rubber Co.*, 749 F.2d 707, 223 USPQ 1264 (CAFC 1984).

Kobayashi et al. Does Not Disclose the Isolation of Autologous Bone Marrow-Mononuclear Cells As Required by Claim 1 Step (a)

Applicants respectfully traverse the Examiner's rejection and maintain that Kobayashi et al. does not disclose each and every element of the claimed method of forming new blood vessels in tissue in a subject.

Claim 1 recites a method of forming new blood vessels in tissue in a subject which comprises: (a) isolating autologous bone marrow-mononuclear cells from the subject; and (b) transplanting locally into the tissue an effective amount of the autologous bone-marrow mononuclear cells, resulting in formation of new blood vessels in the tissue.

Kobayashi et al. use bone marrow cells labeled with a fluorescent dye for injection into ischemic heart, however, Kobayashi et al. do not isolate the mononuclear cells from the bone marrow, as required by the claimed method. Since Kobayashi et al. do not teach all of the elements of the claimed method, Kobayashi et al. do not anticipate claims 1-8, 10, 12-13, 15-16, 18-21, 23-28, 30, 32-34, 36-37, 39-40, 42, 44-45, 47-49 and 53-54.

Accordingly, applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 102(a).

Shintani et al.

Claims 1-2, 5-8, 10-15, 18-21, 23-24, 27-30, 32-33, 36-37, 44-48, and 53-54 have been rejected under 35 U.S.C. § 102(a), as being anticipated by Shintani et al. (Circulation 1999 Nov 2; 100(18):I.406, Abstract).

Shintani et al. Does Not Disclose Transplanting Autologous Bone-marrow Mononuclear Cells into Heart Muscle

Applicants respectfully traverse the Examiner's rejection and maintain that Shintani et al. does not disclose each and every element of the claimed method of forming new blood vessels in tissue in a subject.

The elected species of the treated tissue type of the claimed methods is heart muscle. Shintani et al., describe intramuscular transplantation of bone-marrow derived mononuclear cells into the adductor muscle of ischemic hindlimb. Cardiac muscle differs from skeletal muscle in structure and function: cardiac muscle is made up of striated fibers that function in

long-term rhythmic contraction and is not under voluntary control, whereas, an adductor muscle is a skeletal muscle, which has light and dark bands, is made of elongated multinuclear fibers and is found in muscles under voluntary control attached to the skeleton. Since Shintani et al. do not teach all of the elements of the claimed method, Shintani et al. do not anticipate claims 1-2, 5-8, 10-15, 18-21, 23-24, 27-30, 32-33, 36-37, 44-48, and 53-54, which are directed to heart muscle.

Accordingly, applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 102(a).

In view of the foregoing amendments and remarks, it is firmly believed that the subject invention is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

Dated: January 15, 2003

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